

Analytical Problems with the Evaluation of Human Exposure to Fluorides from Tea Products

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Abstract Tea (*Camellia sinensis*) is a plant exhibiting high tendency for accumulation of fluorides. Up to 98 % of fluorides are accumulated in the leaves used for the preparation of widely consumed tea infusions. Fluorides from tea leaves are released into infusions, which make the second after drinking waters important source of fluorine for humans. A narrow margin between acceptable fluoride concentration and health risk requires a reliable examination of a large variety of tea products for fluoride contents. Large differences between the published results obtained by the use of different analytical techniques have stimulated studies on possibilities and limitations of different analytical procedures used for fluoride quantification in tea infusions. The applicability of ion selective electrode (ISE) and ion chromatographic (IC) methods to the determination of fluorides in the presence of aluminium, the element highly accumulated by tea trees affecting the amount of free fluoride ions occurring in tea infusions, was examined. The efficiency of different total ionic strength adjusting buffer (MES with citrates or tartrates and citrate) for the elimination of aluminium interference with fluoride detection by ISE method was compared. The applicability of three different chromatographic columns, Metrosep A Supp 4 and Metrosep A Supp 5 (both from Metrohm) and IonPac AS9-HC from Dionex, to the isolation of free fluoride signals from the signals of interfering species, including acetates and formates, which can occur in tea samples, were examined. The contents of fluoride in various tea commercial products evaluated by ISE and IC methods applied are compared.

Keywords Tea · Fluoride · Aluminium · ISE method · Ion chromatography

Introduction

Fluoride, a monovalent F^- anion, is a reduced form of fluorine. Fluoride makes the predominant form of fluorine, one of the most chemically and physiologically active elements occurring in nature. Fluorine exhibits both beneficial and toxic effects to human body. Moderate levels of the element are beneficial for preventing dental caries and development of strong bones. A correlation between the rates of dental caries and fluoride concentrations in consumed drinking waters has been known since 1940s (Hardisson et al. 2003). Fluorides can improve bone mineralisation by substitution of hydroxyl groups in hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ involved in building of skeletal bone, generating fluoroapatite, $Ca_{10}(PO_4)_6OHF$ and $Ca_{10}(PO_4)_6F_2$, of reduced crystal volume, increased structural stability and decreased mineral solubility (Ozsvath 2009). This can result in the reduction of a risk of osteoporosis. Adverse effects can occur at higher exposure to the element. Disorders in enamel formation resulting in large gaps in its crystalline structure, mottling of the tooth surface and dental fluorosis are characteristic for fluoride overdose. The excess of fluorides can generate significant disruptive health effects, among them skeletal fluorosis and increased rates of bone fractures; decreased birth rates; increased risk of cancer, in particular bone and blood cancer; increased rates of urolithiasis (kidney stones); and neurotoxicity are the most dangerous (Hardisson et al. 2003; Ozsvath 2009; Yi and Cao 2008). Recent data on negative effects of the excess of fluorides on biological activity of proteins and enzymatic systems, resulting, e.g. in oxidative stress, modification of gene expression, inhibition of immune systems and premature ageing of human body (Barbier et al. 2010), generate urgent question on the real human exposure to

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fluorides. The necessity of the examination of a large variety of fluoride sources for humans is additionally intensified by a narrow margin between the acceptable fluorine concentration range in human body and health risk.

Humans can uptake fluorides from various food products. Dietary recommendations for fluorides, between 1.5 and 4 mg per person per day for adults, 0.1 and 1 mg per person per day for the first year of life, 0.5 and 1.5 mg per person per day for the next 2 years and up to 2.5 mg per person per day for 12-year-old children, have been established to avoid element overdosing (Hardisson et al. 2003). Widely consumed tea infusions are considered the second after drinking waters most important source of fluorides. Dental products, in particular toothpastes treated with fluorides against caries, air contaminated from industrial sources, some medicines and cosmetics can also make the sources of fluorides and require attention.

The problem of the content of fluorides in a large variety of tea products used for the preparation of infusions is of great importance for the estimation of human exposure to the element. In 2004, the total quantity of various teas consumed around the world was estimated to 3×10^9 kg/year (Lu et al. 2004). Tea trees exhibit a high tendency for bioaccumulation of fluorides, mainly (up to 98 % of the total amounts) in the leaves. Fluoride in tea trees origins from soils, waters and air. The content and accumulation level of fluorides in tea products relate pH of soils, maturity of the leaves used and the complexity of technological process applied for the production of various tea types and forms. The optimum range of soil pH for tea plants is 5–5.6. Lower pH facilitates the accumulation of fluorides (Fung and Wong 2002). The content of fluorides in tea products increases with maturity of leaves. Brick and Bianxiao tea types produced in some regions, e.g. in China, made of old leaves exhibit the highest fluoride content, 491.8 mg kg^{-1} (a mean value) (Cao et al. 1998) and up to 708 mg kg^{-1} (Shu et al. 2003). Black teas produced with the application of a fermentation step are generally considered the sources of higher amounts of fluorides than non-fermented green teas (Malinowska et al. 2008; Shu et al. 2003). The concentrations of fluorides in tea infusions also depend on tea form (stick leaves, granules or bags) used, brewing time and repeatability. Substantial effect of the tea form on the content of fluorides in aqueous extracts has been reported (Koblar et al. 2012; Malinowska et al. 2008). About 2.5 higher concentrations in tea infusions prepared from bags as compared with stick-shaped teas were determined (Koblar et al. 2012). An increase in brewing time results in higher amounts of fluorides passing into infusions (Fung et al. 2003; Malinowska et al. 2008). Higher fluoride amounts are also released under repeated infusions compared with continuous method. A logarithmic function adequately describes the efficiency of

fluoride leaching from tea products by the use of repeated infusions (Koblar et al. 2012).

Fluoride uptake can be directly affected by a kind of water used for the preparation of tea infusions. Fluorides occurring in the water increase their final amount entering human body, although there are data on the possibility of absorption of fluoride ions from high-fluoride containing waters by tea leaves, which can result in their lower concentrations in infusions than in the water used (Kjelleve et al. 2006). Hardness of water can influence the efficiency of fluoride extraction from tea products. Over 90 % and only 75 % extraction of fluoride were reported when using soft and hard water, respectively, under similar other conditions applied (Kalayci and Somer 2003).

The contents of the other mineral components in tea products, in particular those exhibiting tendency for the formation of strong complexes with fluoride ions, can affect speciation and bioavailability of the element. Substantial attention is paid to aluminium, which is one of the major mineral constituents of tea products. The problem of the interaction of aluminium with fluoride ions is particularly important taking into consideration high tendency of tea trees for its bioaccumulation. Tea leaves are considered a hyper-accumulator of aluminium. They can absorb up to $30,000 \text{ mg kg}^{-1}$ of aluminium (Matsumoto et al. 1976; Shu et al. 2003). The effectiveness of leaching of aluminium from commercial black tea products into infusions has been evaluated to 2.6–63.3 % (Szymczycha-Madeja et al. 2012). Aluminium can largely affect speciation of fluoride ions in tea infusions owing to ease formation of a variety of Al-F_n^{3-n} (AlF_2^+ , AlF_2^+ , AlF_3 , AlF_4^- , AlF_5^{2-} and AlF_6^{3-}) complexes (Corbillon et al. 2008; Jackson et al. 2002). The occurrence of some organic complexing agents, e.g. polyphenols, in the examined sample can influence the amount of aluminium involved in the formation of the complexes with fluoride ions. According to literature data substantial (10–90 %) amounts of aluminium can be bound by organic tea components (Flaten 2002; Kralj et al. 2005).

Detail studies of the Al–F system have shown that the distribution of the Al–F complexes depends on the amount of aluminium as well as fluoride ions, pH and a total ionic strength of the solution. Acidity of the solution strongly affects the form and stability of various Al–F complexes (Corbillon et al. 2008; Jackson et al. 2002; Li 2003). In general, an increase in pH facilitates the dissociation of the complex species occurring at $\text{pH} \leq 7$. At $\text{pH} 8$ – 9 , all fluorides can exist as free anions. A sharp decrease in the amount of free ions takes place with pH decreasing. The occurrence of only 21.35 % of free F^- anions and 60.40 % of AlF_2^+ complex was reported at $\text{pH} 6$ (Jackson et al. 2002). The AlF_2^+ and AlF_3 complexes were identified as the main species at $\text{pH} 4$ (Li 2003).

The complex equilibria of aluminium–fluoride system require special attention when analytical procedure applied for the evaluation of the total fluoride content in the examined sample is sensitive only to free F^- ions. Such situation takes place in the case of the use of LaF_3 ion selective electrode (ISE) for potentiometric determination of fluorides in matrices of high amounts of aluminium. The introduction of various aluminium masking agents into a total ionic strength adjustment buffer to minimise the interfering effect is widely applied. Acetate, citrate, tartrate and morpholinethanesulfonic acid (MES) based buffer are most often used for potentiometric determination of fluoride ions (Borjigin et al. 2009; Cao et al. 1998; Colina et al. 1990; Fouskaki et al. 2003; Rietjens 1998; Vickery and Vickery 1976). Numerous factors affecting the effectiveness of the decomposition of Al–F complexes, among them acidity, fluoride and aluminium concentrations, as well as the concentration of the buffer used, make the choice of the appropriate analytical conditions for the examination of the complex samples a fundamental for the reliability of the results. The applicability of potentiometric method is limited to pH 5–8 (Williams 1979). The occurrence of HF and HF_2^- species and interfering effect from OH^- anions at lower and higher pH, respectively, can affect the values of fluoride ISE signals. A reliability of the results depends on the complexity of the examined samples. Inconsistencies in fluoride contents determined by potentiometric and the other methods in analysis of more complex, including food and beverage, samples have recently been reported (Martínez-Mier et al. 2011; Whitford 2010). Common application of ISE method for standardisation of fluoride content in tea-certified reference materials substantially limits their use for the evaluation of the reliability of the results obtained by different methods.

Analytical techniques that offer the possibility of fluoride detection at pH favorable for quantitative leaching of free F^- anions from various complex species can be advantageous to potentiometry. Such conditions are available when using ion chromatography (IC) for the determination. The $Na_2CO_3+NaHCO_3$ buffer of pH about 10 is generally used as a standard mobile phase in IC methods to determine anionic species, including free fluorides (Weiss 2004). Such conditions facilitate complete releasing of fluoride ions from the complexes with aluminium. Additionally, ion chromatography allows the detection of fluoride in the presence of the other inorganic and small organic anions present in the examined solution (Fig. 1). Till now, the IC technique has relatively been rarely used for the evaluation of fluoride content in tea products. However, higher fluoride amounts in tea infusions determined by IC methods compared with those by potentiometry turn the attention (Kumar et al. 2008; Michalski 2006). Large differences between the results obtained by IC and ISE methods, as well as earlier data on higher amounts of fluoride in tea products

determined by the other method (Whitford 2010), generate the necessity of detailed further studies on the reliability of the results obtained by different techniques.

Attempts to compare the possibilities and limitations of ISE and IC methods for the determination of fluoride in the presence of aluminium are described in the present work. The results obtained by the use of both techniques in analysis of various tea products are presented.

Experimental

Apparatus

The 883 Basic IC Compact System from Metrohm AG (Herisau, Switzerland) consisting of an MSM II (Metrohm Suppressor Module) suppressor, conductometric and UV–vis detectors, was used. Anion separation was carried out on three columns: Metrosep A Supp 4 (250×4 mm) and Metrosep A Supp 5 (150×4 mm), both from Metrohm; and IonPac AS9-HC (250×4 mm) from Dionex, USA. The volume of a sample injection loop was 20 μ L. The MagIC Net 2.3 (Metrohm) software was used for data acquisition and evaluation of chromatograms.

The potentiometric detection was carried out using the fluoride ion-selective electrode (Detektor s.c., Poland) and EMF16 Interface meter (Lawson Labs, Inc., USA). An Ag/AgCl, KCl electrode (RL-100, Hydromet, Poland) was used as an internal reference electrode. All potentials were measured at $22\pm1^\circ C$. Equilibria were achieved after 10 min.

Filters of pore size of 0.45 μ m MCE (Fisherbrand) and IC-RP (C18) from Metrohm were used for sample filtration throughout IC experiments.

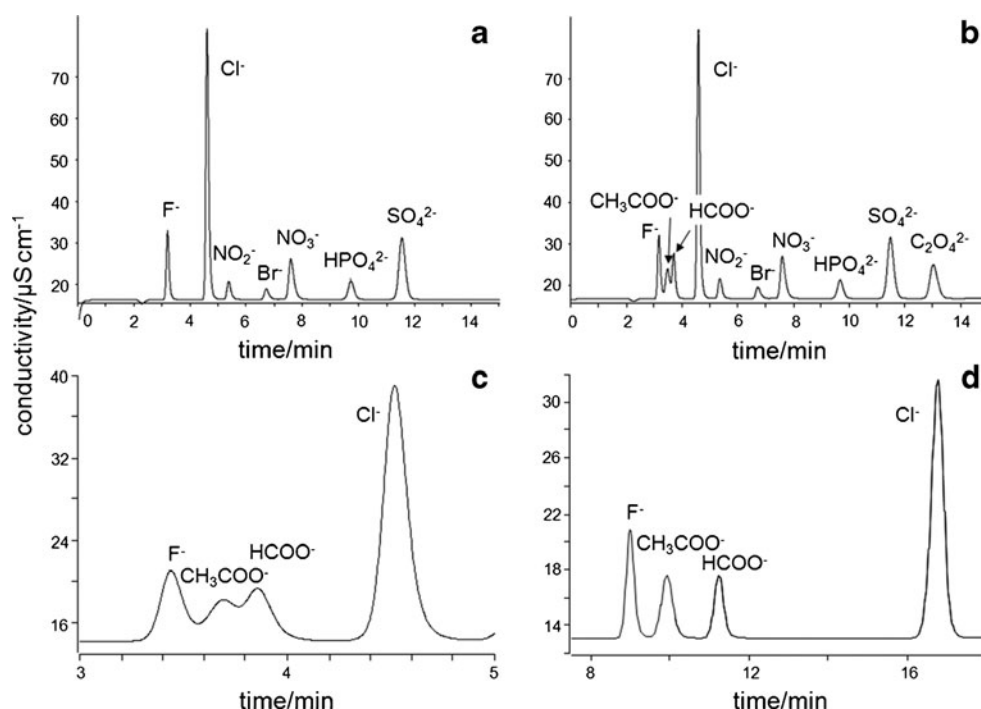
Reagents

All reagents used in the work were of analytical grades. Water purified in a Millipore Elix3/Simplicity UV system (a specific resistance, $>18.2\text{ M}\Omega\text{cm}$) was used in all experiments.

Standard solutions of inorganic [fluoride, chloride, nitrate (III), bromide, nitrate(V), phosphate and sulphate] anions prepared from high purity sodium salts and organic (acetate, formate and oxalate) anions prepared from high purity acids, both dedicated to IC (from Fluka) and containing 1,000 mg L^{-1} of each anion, were used. Multi-ion standard solutions containing inorganic anions within the concentration (mg L^{-1}) ranges: F^- (0.4–10), Cl^- (0.8–75), NO_2^- (0.4–10), Br^- (0.4–10), NO_3^- (0.6–50), PO_4^{3-} (0.8–75) and SO_4^{2-} (0.6–25) were used for the calibration procedure.

Sodium carbonate (Na_2CO_3) and sodium hydrogen carbonate ($NaHCO_3$) from Fluka were used for the preparation of the eluents: (1) $1.8\text{ mmolL}^{-1} Na_2CO_3+1.7\text{ mmolL}^{-1} NaHCO_3$ (pH=10.2); (2) $3.2\text{ mmolL}^{-1} Na_2CO_3+$

Fig. 1 The ion chromatograms of synthetic mixtures of anions [5 (F^-), 25 (Cl^-), 5 (NO_2^-), 5 (Br^-), 15 (NO_3^-), 15 (PO_4^{3-}), 20 (SO_4^{2-}), 50 (CH_3COO^-), 10 (HCOO^-) and 20 ($\text{C}_2\text{O}_4^{2-}$) mg L^{-1}] under different chromatographic conditions: **a**, **b** Metrosep A Supp 5 Metrohm column, $3.2 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3 + 1.0 \text{ mmol L}^{-1} \text{NaHCO}_3$ (0.7 mL min^{-1}) eluent; **c** Metrosep A Supp 4 Metrohm column, $1.8 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3 + 1.7 \text{ mmol L}^{-1} \text{NaHCO}_3$ (1.0 mL min^{-1}) eluent; **d** IonPac AS9-HC Dionex column, $3.0 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3$ (0.5 mL min^{-1}) eluent



$1.0 \text{ mmol L}^{-1} \text{NaHCO}_3$ ($\text{pH}=10.6$) and (3) $3 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3$ ($\text{pH}=10.8$) used under different chromatographic conditions (Table 1).

Certified multianion standard solution dedicated to IC (from Fluka) containing an aqueous mixture of F^- , Cl^- , Br^- , NO_3^- , PO_4^{3-} and SO_4^{2-} (10 mg L^{-1} each of anions) was used for the evaluation of the accuracy of IC method developed.

Sodium fluoride ($1 \text{ mol L}^{-1} \text{NaF}$) prepared by dissolving of 4.2 g of the reagent (from Fluka) in 100 mL of deionised water was used for the preparation of standard solutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and $10^{-7} \text{ mol L}^{-1} \text{F}^-$) for potentiometric calibration and measurements. Fluoride working solutions were prepared freshly each day and stored in polyethylene bottles.

MES buffer containing 0.1 mol L^{-1} 2-(*N*-morpholino)ethanesulfonic acid monohydrate and 0.02 mol L^{-1} sodium chloride (both from Sigma-Aldrich) was prepared by the dissolution of 21.33 and 1.17 g of the reagents, respectively, in about 800 mL of deionised water, adjusted to $\text{pH}=5.5$ or 6.5 with sodium hydroxide (from POCh, Poland) and diluted to a total volume of $1,000 \text{ mL}$. All solutions submitted to the measurement, of final volume of 50 mL , contained 25 mL of this buffer.

Sodium citrate, 1.25 mol L^{-1} solution, was prepared by the dissolution of 91.91 g of $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ (Chempur, Poland) in 250 mL of deionised water. Ten milliliters of the solution was added to the examined samples prior submitting to potentiometric measurements (50 mL of final volume).

Table 1 Statistical evaluations of the IC results ($n=6$) for fluoride (0.4 , 2.0 , 5.0 and 10.0 mg L^{-1}) obtained under different chromatographic conditions

| Peak area | | Peak height | |
|---|---------|--|---------|
| Concentration \pm SD ($\text{mg L}^{-1} \text{F}^-$) | RSD (%) | Concentration \pm SD ($\text{mg L}^{-1} \text{F}^-$) | RSD (%) |
| Metrosep A Supp 5, Metrohm column, $3.2 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3 + 1.0 \text{ mmol L}^{-1} \text{NaHCO}_3$ (0.7 mL min^{-1}) eluent | | | |
| 0.39 ± 0.01 | 2.6 | 0.40 ± 0.01 | 2.5 |
| 2.00 ± 0.01 | 0.5 | 1.95 ± 0.01 | 0.5 |
| 5.01 ± 0.01 | 0.2 | 4.96 ± 0.04 | 0.8 |
| 9.99 ± 0.04 | 0.4 | 10.05 ± 0.06 | 0.6 |
| IonPac AS9-HC, Dionex column, $3.0 \text{ mmol L}^{-1} \text{Na}_2\text{CO}_3$ (0.5 mL min^{-1}) eluent | | | |
| 0.40 ± 0.01 | 2.5 | 0.40 ± 0.01 | 2.5 |
| 2.00 ± 0.01 | 0.5 | 2.00 ± 0.01 | 0.5 |
| 5.01 ± 0.03 | 0.6 | 5.00 ± 0.03 | 0.6 |
| 10.00 ± 0.02 | 0.2 | 10.00 ± 0.01 | 0.1 |

Citrate buffer containing 0.29 molL^{-1} citric acid (POCh, Poland), 0.71 molL^{-1} sodium citrate (Chempur, Poland) and 1 molL^{-1} ammonium chloride (POCh, Poland) was prepared by the dissolution of 60, 210 and 53.5 g of the reagents, respectively, in about 800 mL of deionised water, adjusted to pH 5.5 or 8.0 with NaOH and NH_3 (both from POCh, Poland) and diluted to a final volume of 1,000 mL.

Tartrate solution containing 1 molL^{-1} of sodium and potassium salt was prepared by the dissolution of 70.56 g of $\text{C}_4\text{H}_4\text{O}_6\text{KNa}\cdot 4\text{H}_2\text{O}$ in 250 mL of deionised water. Ten milliliters of the solution was added to the examined samples (of 50 mL final volume) prior submitting to the measurements.

Aluminium sulphate, 500 mgL^{-1} Al, was prepared by the dissolution of 1.544 g $\text{Al}_2(\text{SO}_4)_3\cdot 18\text{H}_2\text{O}$ (POCH, Poland) in 250 mL of deionised water.

Tea Samples Examined

Black tea samples of various producers, e.g. Lipton, Loyd (Assam, Ceylon, Madras and Yunnan) and Tetley of different form (stick-shaped, granules and paper bag-packages) bought at the local market and the certified tea reference material NCS ZC73014 (NCS Certified Reference Material, China National Analysis Center for Iron & Steel), were examined.

Analytical Procedure

Tea samples were preliminary dried at 60°C for 4 h (tea from bag packages after removing of paper bags). For the infusion, 2 g of tea was treated by 200 mL of boiling deionised water. After 5 min, the sample was filtered, cooled to room temperature, transferred into a 250-mL calibration flask and diluted to final volume with deionised water. The solutions were injected into the chromatographic column after filtration through $0.45\text{-}\mu\text{m}$ membrane filter and IC-RP cartridge. Twenty-five milliliters of the solutions were directly submitted to potentiometric measurements after mixing with 25 mL of the buffer used.

Results and Discussion

The Determination of Fluoride Ions by ISE Technique

In our work, we have examined the effect of aluminium, in a large concentration range (up to $40 \text{ mgL}^{-1} \text{ Al}^{3+}$), on fluoride ($2 \text{ mgL}^{-1} \text{ F}^-$) ISE signals under different pH and different total ionic strength adjusting buffers used. The chosen fluoride concentration relates the element level most often determined in tea infusions. Maximum aluminium concentration examined corresponds to 5 mg g^{-1} in dry tea (2 g sample in 250 mL

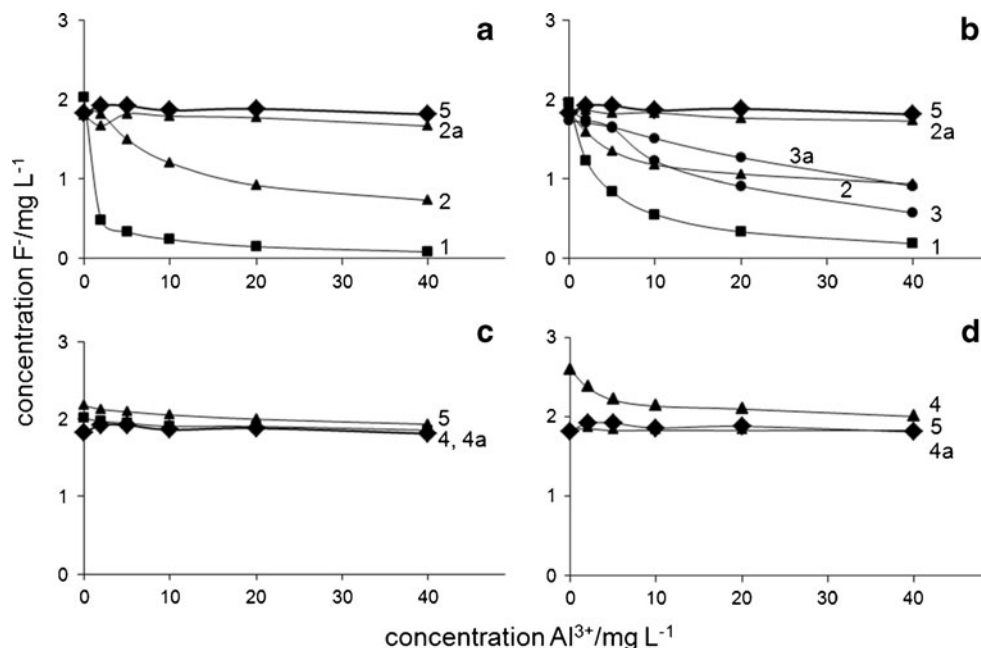
of the infusion). The potentiometric experiments were carried out at different pH of 5.5, 6.5 and 8, covering the fluoride electrode applicability range. Possibilities of three buffers, i.e. MES with citrates or tartrates and citrate (alone), for the elimination of the interfering effects from the metal ions have been examined (Fig. 2). The results were compared with those obtained by the use of the developed IC procedure (described below) allowing the determination of fluoride ions at pH 10 facilitating the dissociation of Al–F complexes. As can be seen in Fig. 2 (curve 5), the IC method used is practically free from aluminium interference.

A sharp decrease in the results for fluorides with the increase in Al^{3+} concentration in the examined solutions is observed for the ISE method when using MES (0.05 molL^{-1} MES + 0.01 molL^{-1} NaCl) buffer alone (Fig. 2a, curve 1). The interfering effect from aluminium can be decreased by the introduction of citrates into the solution containing MES buffer (Fig. 2a, curve 2). The beneficial effect of citrates increases in time. After 24 h, the amounts of fluorides evaluated by ISE method (Fig. 2a, curve 2a) correspond to those directly obtained by the IC procedure. The use of citrate buffer is also advantageous to tartrate used for aluminium complexation (Fig. 2b, curves 3 and 3a). The experiments have shown that the effect of aluminium on fluoride ISE signals is completely eliminated when citrates are added to the examined solution prior to the MES buffer or a mixture MES+citrates is directly used. We have additionally established that the use of citrates (ammonium citrate buffer has been examined) alone, without MES, is sufficient for the elimination of aluminium interference on fluoride signals (Fig. 2c, curve 4). No changes in the registered signals were observed after 24 h in solutions containing $2\text{--}20 \text{ mgL}^{-1}$ of fluoride ions in the examined concentration range of aluminium.

Twenty-five milliliters of ammonium citrate (0.29 molL^{-1} citric acid and 0.71 molL^{-1} sodium citrate in mixture with 1 molL^{-1} ammonium chloride), adjusted to the required pH with NH_3 and NaOH, buffer added to 25 mL of tea infusion (0.5 molL^{-1} final citrate concentration) and pH 5.5 were chosen for the determination of fluoride content by ISE method. An acidity of the examined tea infusions amounted to 5.1 ± 0.1 . Attempts to enhance pH of the solutions to slightly alkaline (pH = 8 was examined) failed owing to interfering effect from OH^- ions on the fluoride electrode signals (Fig. 2d, curve 4). Analytical fluoride concentration range could be determined under the conditions used was of $1 \times 10^{-6}\text{--}0.1 \text{ molL}^{-1}$. The detection limit taken at the point of intersection of two extrapolated segments of the calibration graph was $9.5 \times 10^{-7} \text{ molL}^{-1}$.

The results for fluoride contents in the examined tea samples by the ISE method under the conditions used are presented in Table 2. The recovery of fluoride ions introduced as

Fig. 2 The effect of a kind of buffer: MES (0.05 mol L⁻¹)+NaCl (0.01 mol L⁻¹)—curve 1; MES (0.05 mol L⁻¹)+NaCl (0.01 mol L⁻¹)+citrates (0.25 mol L⁻¹)—curve 2 (after 24 h, curve 2a); MES (0.05 mol L⁻¹)+NaCl (0.01 mol L⁻¹)+tartrates (0.2 mol L⁻¹)—curve 3 (after 24 h, curve 3a); citrates (0.5 mol L⁻¹)+NH₄Cl (0.5 mol L⁻¹)+NH₃, NaOH—curve 4 (after 24 h, curve 4a); and **a**, **c** pH 5.5; **b** pH 6.5; **d** pH 8.0 on the determination of fluoride (2 mg L⁻¹) in the presence of different amounts of Al³⁺ ions by ISE—curves 1–4, and IC (Metrosep A Supp 5 Metrohm column, 3.2 mmol L⁻¹ Na₂CO₃+1.0 mmol L⁻¹ NaHCO₃ (0.7 mL min⁻¹) eluent)—curve 5, methods



a spike (0.600 mg g⁻¹) was in the range of 94.7–99.2 %. Very good agreement between the obtained results for fluoride content (0.059±0.001 mg g⁻¹) and certified value (0.057±0.015 mg g⁻¹) in tea CRM examined was achieved.

The Determination of Fluoride Ions by IC Technique

Calibration of the IC chromatograph was carried out for two different analytical columns: Metrohm Metrosep A Supp 5 and Dionex IonPac AS9-HC, dedicated to the determination of anionic species in the examined samples. Standard

solutions containing fluoride ions in mixtures with chloride, nitrate(III), bromide, nitrate(V), phosphate and sulphate anions (Fig. 1a) were used for calibration. All these inorganic anions can be identified and determined by IC technique if they occur in the examined tea infusions. The fluoride concentrations in the calibration solutions were in the range of 0.4–10 mg L⁻¹ corresponding their expected amounts in the examined tea samples (about 2 g in 250 mL). Each of calibration solution was run triplicate.

Two calibration curves were generated by plotting (1) the peak areas or (2) peak heights against fluoride concentrations

Table 2 The content of fluoride in tea infusions determined by IC [IonPac AS9-HC Dionex column, 3.0 mmol L⁻¹ Na₂CO₃ (0.5 mL min⁻¹) eluent] and ISE [pH 5.5; ammonium citrate (0.29 mol L⁻¹ citric acid, 0.71 mol L⁻¹ sodium citrate, 1 mol L⁻¹ ammonium chloride, NH₃ and NaOH) buffer] methods (*n*=3)

| Tea | Geographical origin | Shape | Concentration±SD | | | |
|--|---|----------|--------------------|-----------|--------------------|-------------|
| | | | mg L ⁻¹ | | mg g ⁻¹ | |
| | | | IC | ISE | IC | ISE |
| Assam | India | Sticks | 2.43±0.05 | 0.99±0.02 | 0.300±0.006 | 0.122±0.003 |
| Yunnan | China | Sticks | nd | 0.55±0.01 | nd | 0.068±0.002 |
| Madras | India | Sticks | 4.65±0.06 | 2.65±0.07 | 0.575±0.009 | 0.328±0.010 |
| Ceylon | Sri Lanka | Sticks | nd | 0.52±0.01 | nd | 0.064±0.002 |
| | | Bags | 3.77±0.11 | 1.76±0.04 | 0.462±0.008 | 0.216±0.001 |
| Assam | India | Bags | 4.39±0.05 | 2.14±0.02 | 0.507±0.005 | 0.247±0.003 |
| Mixture | Different countries (Kenya, Indonesia, India) | Sticks | 3.27±0.16 | 1.44±0.07 | 0.403±0.016 | 0.177±0.009 |
| | | Granules | 4.27±0.02 | 2.09±0.04 | 0.528±0.001 | 0.259±0.004 |
| | | Bags | 4.40±0.08 | 2.31±0.09 | 0.523±0.009 | 0.274±0.007 |
| Tea certified reference material (NCS ZC73014); certified (ISE method) content of fluoride: 0.057±0.015 mg g ⁻¹ | | | 2.17±0.01 | 0.49±0.01 | 0.269±0.002 | 0.059±0.001 |

nd not determined

in the injected standards (Fig. 3). The use of peak height for the evaluation of the analyte content (the option available in the software) can be more accurate in case of difficulties with complete resolution of IC signals of the analyte and the other species in analysis of more complex samples (Bicking 2006; Weiss 2004). Statistical evaluation of the results for standard solutions used obtained by the application of both columns and different integration systems is presented in Table 1. The results obtained when using both integration systems are similar in terms of precision and accuracy. The accuracy of the procedure applied was evaluated in analysis of Certified Multianion Standard Solution (Fluka) containing $10 \text{ mg L}^{-1} \text{ F}^{-}$ in mixture with the other inorganic ions. The recovery of fluoride was in the range of 101–102 % and 102–104 % when using Dionex IonPac AS9-HC and Metrosep A Supp 5 columns, respectively (six independent analyses were carried out and peak height mode was used for calculation).

When examining tea infusions, the occurrence of small organic anions, e.g. acetates and formates, requires taking into consideration owing to possible interferences with fluoride detection. Oxalate anions occurring in the examined solution can also be simultaneously detected under the IC procedure. As can be seen in Fig. 1b, oxalate signals do not interfere with fluoride determination owing to substantial

difference in the retention times of both anions. Our experiments have shown that the possibility of isolation of fluoride signal from acetate and formate depends on the conditions used. The kind of chromatographic column, eluent composition and flow rate substantially affect the efficiency of isolation of fluoride and the registered organic anion signals. We have examined the applicability of both examined columns, Metrosep A Supp 5 and IonPac AS9-HC, and additionally Metrosep A Supp 4, widely used for the determination of anionic species, to the determination of fluoride ions in the presence of acetates and formates (Fig. 1). Better conditions for the isolation of the signals of particular anions were achieved by the use of Dionex IonPac AS9-HC column (Fig. 1d) than both Metrohm, Metrosep A Supp 5 (Fig. 1b) and Metrosep A Supp 4 (Fig. 1c), columns. A Dionex IonPac AS9-HC column and $3.0 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$ eluent at 0.5 mL min^{-1} flow rate were chosen for the determination of fluoride ions in the examined tea infusions. The detection limit calculated as three times of standard deviation of the results for solution containing the lowest (0.1 mg L^{-1}) analyte concentration could be determined and 5 mg L^{-1} of formate and acetate anions, under the condition used, was of $0.02 \text{ mg L}^{-1} \text{ F}^{-}$.

Figure 4 shows the example of chromatograms of tea sample (Madras, India, stick shaped) registered when using two columns [Metrohm Metrosep A Supp 5 (Fig. 4a) and Dionex IonPac AS9-HC (Fig. 4b)] under the optimum conditions established for particular columns. The use of IonPac AS9-HC Dionex column allows identification of fluoride signal at the slope of higher peak of unknown species totally overlapping of fluoride signal when using Metrohm Metrosep A Supp 5 column. Similar situation was observed in the analysis of the certified tea reference material (NCS ZC73014) (Fig. 5). The smaller peak identified when using IonPac AS9-HC Dionex column and conductometric detection (Fig. 5a, curve 1) well corresponds with the spike of free fluoride ions (Fig. 5a, curve 2). The results were compared with those obtained when coupling UV–vis detector with IC chromatograph (Fig. 5b). No absorption of radiation by free fluoride ions (Fig. 5b, curve 3) and clearly registered signal of the other species (Fig. 5b – curve 1) suggest a complex (molecular) composition of the latter capable of absorption of radiation. The UV–vis spectrum of tea infusion examined does not change after introduction of free fluorides (3.3 mg L^{-1} spike) (Fig. 5b, curve 2). A question arises about the composition of a molecular species interfering with the signals of free fluoride ions and, in particular, if that species can bind of fluorides. The explanation requires further detail studies on chemical composition of the molecular species by techniques of higher identification potentials, e.g. ESI-MS.

The results for the content of fluoride in the examined tea infusions obtained by IC method used are given in Table 2. It should be noted that the results (mg g^{-1}) given for dry teas

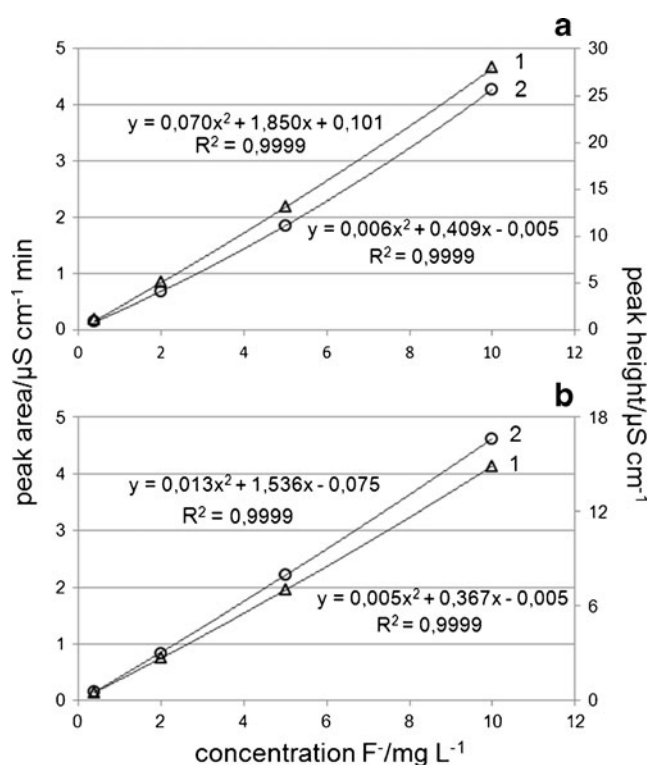
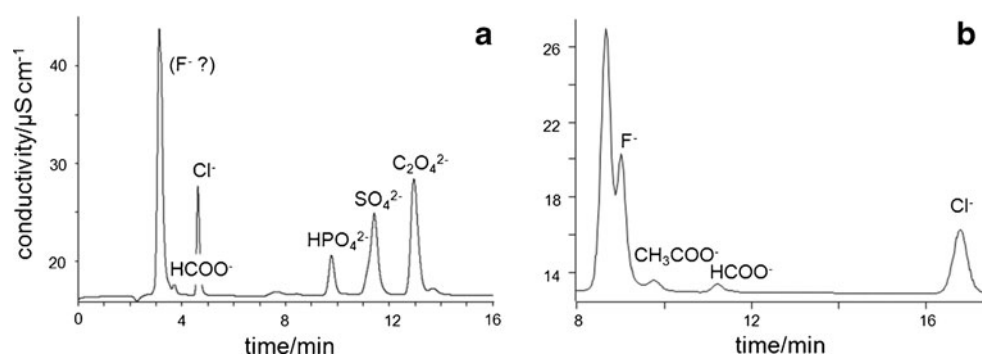


Fig. 3 Calibration curves for fluoride determination by IC methods using (1) peak area and (2) peak height: **a** Metrosep A Supp 5 Metrohm column, $3.2 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3 + 1.0 \text{ mmol L}^{-1} \text{ NaHCO}_3$ (0.7 mL min^{-1}) eluent. **b** IonPac AS9-HC Dionex column, $3.0 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$ (0.5 mL min^{-1}) eluent

Fig. 4 The ion chromatograms of black (Madras, sticks) tea infusion under different chromatographic conditions: **a** Metrosep A Supp 5 Metrohm column, $3.2 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3 + 1.0 \text{ mmol L}^{-1} \text{ NaHCO}_3$ (0.7 mL min^{-1}) eluent; **b** IonPac AS9-HC Dionex column, $3.0 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$ (0.5 mL min^{-1}) eluent



relate the amounts of fluoride leached from the examined samples under the conditions used [5 min brewing time and the chosen ratio of the sample (2 g) weight and the water volume (200 mL) used for extraction]. Longer extraction time results in the increase in fluoride amounts passing into tea infusions. The amount of fluoride extracted by water under the conditions used did not increase after 20 min brewing time.

Higher (about twice) concentrations of fluorides in the examined tea infusions have been determined when using the developed IC procedure as compared with ISE method (Table 2). This can confirm earlier data on possible higher amounts of fluorides occurring in tea products than commonly determined by ISE procedures (Whitford 2010). It can be noted that our results ($0.30\text{--}0.57 \text{ mg g}^{-1}$) are substantially lower as compared with those, $1.20\text{--}3.20 \text{ mg g}^{-1}$ (Kumar et al. 2008) and $12.24\text{--}21.23 \text{ mg g}^{-1}$ (Michalski 2006), earlier reported for IC technique when the complexity of chromatographic signal occurring at the retention time characteristic for fluoride ions was not taken into consideration. The problem of the interference with fluoride detection by IC technique seems complicated. In our experiments, in the analysis of two tea samples examined (Yunnan, China, sticks and Ceylon, Sri Lanka, sticks), the signals of free fluoride ions were not isolated under the conditions used and due to that the fluoride contents in such samples were not evaluated by IC procedure applied (Table 2).

The accuracy of our results was evaluated by a standard addition method and in analysis of certified reference material (NCS ZC73014). The recoveries of fluoride evaluated by a standard (0.600 mg g^{-1} spike introduced) addition were in the range of 94.8–99.2 %. Substantially higher ($0.269 \pm 0.002 \text{ mg g}^{-1}$) amounts of fluorides as compared with the certified value ($0.057 \pm 0.015 \text{ mg g}^{-1}$) were determined in analysis of CRM examined by the IC procedure applied in the work (Table 2). Such situation directly shows an inconsistency between the results obtained by both techniques and can confirm earlier literature data (Whitford 2010) on higher than generally determined by ISE methods fluoride content in tea samples. In our opinion further detail studies are required for explanation the reliability of the results obtained by different analytical techniques.

Conclusions

The evaluation of human exposure for fluorides, of both natural and anthropogenic origin, is important due to confirmed negative health effects from the element. Widely consumed tea infusions ranks second after drinking waters source of fluorides for humans. A large variety of tea products, numerous factors affecting the fluoride content and speciation make the estimation of real fluoride exposure a challenge. Potentiometric ISE technique is commonly used

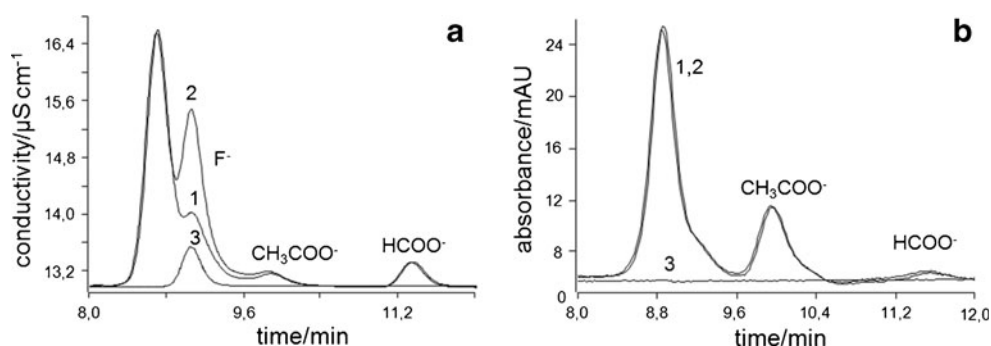


Fig. 5 The ion chromatograms [IonPac AS9-HC Dionex column, $3.0 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$ (0.5 mL min^{-1}) eluent] of the infusion of tea certified reference material, NCS ZC73014 (directly analysed—curve

1; spiked (0.83 mg F^{-} into 250 mL solution)—curve 2), and fluoride (0.4 mg L^{-1}) standard solution—curve 3. **a** Conductometric and **b** spectrophotometric ($\lambda=210 \text{ nm}$) detection

for the determination of fluorides in various samples. The technique is, however, sensitive only to free F^- ions. The effectiveness of the conversion of fluorides into free ions, as well as the conditions used for the detection, requires attention. The application of citrates for the complexation of aluminium that can occur in tea samples at high amounts and can bind fluoride into strong Al–F complexes has been found the most suitable for the ISE detection. Ion chromatography is a technique widely used for the determination of anionic, inorganic and small organic species in a large variety of matrices. The application of IC to the determination of fluorides in tea infusions requires conditions ensuring the isolation of free fluoride signals. In the analysis of tea samples, strong interference from unknown species with fluoride detection can occur. A final conclusion on the applicability of the IC technique to the determination of fluorides in tea products requires further detailed studies on the reliability of the results due to difficulties with complete isolation of free fluoride ion signal. The use of chromatographic columns of higher potential for resolving signals of free fluoride and the other species occurring in tea samples, as well as analytical techniques allowing the identification of the species interfering with IC fluoride signals, would be advantageous. This can provide an explanation of the inconsistencies in the results obtained by different analytical techniques and a more reliable evaluation of the total human exposure to fluorides.

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